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ORIGINAL PAPER

Electrochemical oxidation and determination of antiretroviral drug nevirapine based on uracil-modified carbon paste electrode

Fenfen Zhang · Li Li · Liqiang Luo · Yaping Ding · Xiao Liu

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Abstract A novel uracil covalently grafted carbon paste electrode (Ura/CPE) based on electro-deposition of uracil on CPE was prepared for the quantitative determination of nevirapine. The records of electrochemical impedance spectroscopy (EIS) and cyclic voltammograms (CV) in $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ solution illustrated that uracil grafted on CPE efficiently decreased the charge transfer resistance value of electrode and improved the electron transfer kinetic between analyte and electrode. The electrochemical properties of Ura/CPE towards the oxidation of nevirapine were investigated by cyclic voltammetry and differential pulse voltammetry (DPV) in 0.1 M NaOH. The effects of pH and scan rates on the oxidation of nevirapine were studied. The results indicated the participation of the same protons and electrons in the oxidation of nevirapine, and the electrochemical reaction of nevirapine on Ura/CPE is an adsorption-controlled process. Under optimized conditions, the linearity between the oxidation peak current and nevirapine concentration was obtained in the range of 0.1–70.0 μM with detection limit of 0.05 μM and the sensitivity of 2.073 μA mM^{-1} cm^{-2} ($S/N = 3$). The proposed method was also successfully applied to detect the concentration of nevirapine in human serum samples.

Keywords Uracil · Carbon paste electrode · Nevirapine · Cyclic voltammetry · Differential pulse voltammetry

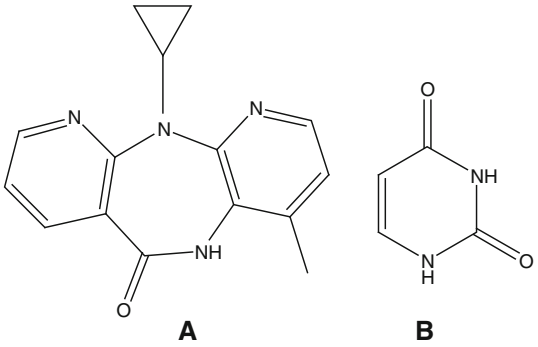
F. Zhang · Y. Ding
School of Materials Science and Engineering, Shanghai University, Shanghai 200444, People's Republic of China

L. Li · L. Luo · Y. Ding (✉) · X. Liu
Department of Chemistry, College of Sciences, Shanghai University, Shanghai 200444, People's Republic of China
e-mail: wdingyp@sina.com

1 Introduction

The HIV reverse transcriptase is an important antiviral target for the chemotherapy of AIDS because of its key role in virus replication [1]. Non-nucleoside reverse transcriptase inhibitors are anti-HIV compounds licensed for the treatment of AIDS. They attach themselves to reverse transcriptase, interact with an allosteric site located at a short distance from catalytic site of enzyme [2] and prevent the enzyme from converting RNA to DNA [3]. Nevirapine, 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido [3,2-b:2′,3′-e][1, 4]diazepin-6-one (Scheme 1a) is the first member of the non-nucleoside reverse transcriptase inhibitors [4]. Nevirapine displays a butterfly-like conformation, which is verified from the crystalline structure of the pure compound [5] and some complexes with the HIV-1 reverse transcriptase [6–8]. It binds directly to allosteric site on reverse transcriptase and inhibits both the RNA- and DNA-dependant DNA polymerase activities. Several researchers suggested that this conformation is related to the degree of affinity of the drug and to the probability of appearance of viral resistance [9, 10].

Various techniques have been developed for the analysis of nevirapine, including high-performance liquid chromatography (HPLC) [11], matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) [12], liquid chromatography–mass spectrometry (LC–MS/MS) [13], micellar electrokinetic chromatographic (MEKC) [14], capillary electrophoresis [15], high-performance thin layer chromatography (HPTLC) [16], high-performance liquid chromatography–ultraviolet–visible spectroscopy (HPLC–UV) [17], and radio-immunoassay [18]. In spite of the great success of the above-mentioned methods, their high costs, time-consuming and complicated operations limit their applications in routine laboratories.



Scheme 1 The structure of nevirapine (**a**) and uracil (**b**)

Electrochemical method is an alternative approach with the advantages of simple, quick and low cost. Until now, electrochemical determinations of nevirapine were almost based on adsorptive stripping voltammetry on thin-film mercury electrode [19]. However, the utilization of the mercury electrodes would contaminate the environment because of their environmental toxicity. Therefore, it is highly advisable to establish nontoxic and selective electrochemical methods for the determination of nevirapine.

Recently, modified electrodes for the determinations of drug molecules have attracted more and more attention because the modifiers could greatly enhance/increase the responds and selectivity for analytes [20–24]. Organic small molecules, polymer, metal nanoparticles, carbon nanotubes, and graphene were often used as modifiers to fabricate the modified electrodes [22–25]. Among them, organic small molecules grafted electrodes showed super stability, quick response and high sensitivity. Uracil (Scheme 1b) is one of the five main nucleobases found in the nucleic acids DNA and RNA, which readily undergoes regular reactions including oxidation, nitration, and alkylation. As previous reported [26, 27], it would be oxidized to radicals containing oxygen and may be covalently grafted on an anodically activated carbon electrode by forming C–O covalent linkages. To the best of our knowledge, uracil-grafted carbon paste electrode (CPE) for determination of nevirapine has not been reported.

In this paper, a novel electrochemical sensor was constructed by electrodeposition of uracil on CPE and applied to determine the concentration of nevirapine. Under the optimized conditions, a good linear relationship between the peak current and nevirapine concentration was observed with wider linear range and low detection limit. This method was also successfully used to detect the concentration of nevirapine in human serum samples and the results were satisfied. This proposed sensor exhibited good reproducibility, long-term stability, and fast current response.

2 Experimental

2.1 Apparatus and reagents

All electrochemical experiments were carried out on a CHI 660D electrochemical workstation (Shanghai Chenhua Co. Ltd., China) with a conventional three-electrode system consisting of a Pt wire counter electrode, a saturated calomel reference electrode (SCE) and a uracil-grafted carbon paste electrode as working electrode. All potential values given below were referred to the SCE. The electrochemical impedance spectroscopy (EIS) was recorded on Solartron 1255B frequency response analyzer/SI 1287 electrochemical interface (Scribner Associates, Inc.).

All reagents used were of analytical grade unless mentioned otherwise, and double distilled water (DDW) was used throughout. Nevirapine was synthesized according to the published literature [28]. Uracil, graphite powder (spectral reagent), paraffin oil, sodium hydroxide, hydrogen chloride, hydrogen nitrate, and ethanol were purchased from the Sinopharm Group Chemical Reagent Co., Ltd., Shanghai, China. NaOH (0.1 M) solution was chosen as the supporting electrolyte.

2.2 Fabrication of CPE and Ura/CPE

Graphite powder and paraffin oil were mixed at the ratio of 3:1 (w/w) and ground by pestle in the agate mortar for 20 min. And then the mixture was firmly packed into the glass tube (i.d. = 2.6 mm) which had been ultrasonicated in HNO_3, NaOH solution, and DDW in turn. Finally, the copper wire was inserted from another end of the tube. The CPE was polished with a piece of weighing paper and rinsed with DDW.

The Ura/CPE was prepared as described in Ref. [29]. CPE was dipped into 0.1 M phosphate buffer solution (PBS) (pH 7.0) solution containing 1 mM uracil. The electrochemical process was performed at 1.8 V for 30 min for modification. Subsequently, the modified electrode was rinsed with DDW and ultrasonicated for 5 min to remove the physical adsorbed molecules. Finally, the Ura/CPE was carefully washed with DDW and stored in 4 °C for use.

2.3 Experimental measurements

EIS were performed at bare CPE and Ura/CPE in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 M KCl at the condition of an alternating current voltage of 10 mV, a bias potential of 200 mV within a frequency range of 10^{-1}–10^5 Hz. CVs were carried out in quiescent solution at a scan rate of 100 mV s^{-1} in an electrochemical cell filled with 10 mL 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) solution containing 0.1 M KCl and 10.0 mL 0.1 M NaOH,

respectively. DPV were performed in an electrochemical cell filled with 10.0 mL 0.1 M NaOH.

3 Results and discussion

3.1 Electrochemical behavior of nevirapine at bare CPE and Ura/CPE

Uracil was successfully grafted on the surface of electrode and the covalently grafted mechanism was described as Ref. [29] which was shown in Scheme 2. Then, the electrochemical performances of Ura/CPE were investigated in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 M KCl solution by CV and EIS, presented in the form of the Nyquist plot. Figure 1a showed the typical results of CV, it can be observed that a pair of redox peaks with ΔE_p of 244 mV at bare CPE (a), while at Ura/CPE (b), the ΔE_p decreased to be 158 mV with response currents almost three times higher than that at bare CPE, suggesting that uracil capped on electrode increased electron transfer kinetics and enlarged electroactive surface area compared to bare CPE.

EIS was also applied to study the electrochemical properties of Ura/CPE. EIS is an efficient tool to probe the interface properties of electrodes surface. The curve of the EIS includes a semicircular part and a linear part, corresponding to the electron transfer resistance and the diffusion process, respectively. The diameter of the semicircle is usually equal to the electron transfer resistance (R_{et}), which normally reflects the conductivity and the electron transfer process [30]. Figure 1b illustrated the typical results of EIS of the bare CPE (a) and Ura/CPE (b). It can be seen that the electron transfer resistance of bare CPE (1,300 Ω) decreased rapidly on Ura/CPE (80 Ω), indicating that uracil has been modified on the surface of electrode and improved the conductivity and electron transfer process.

Figure 1c displayed the typical CV of bare and uracil modified CPE in 0.1 M NaOH solution without and with 100 μM nevirapine, respectively. As shown in Fig. 1c, no

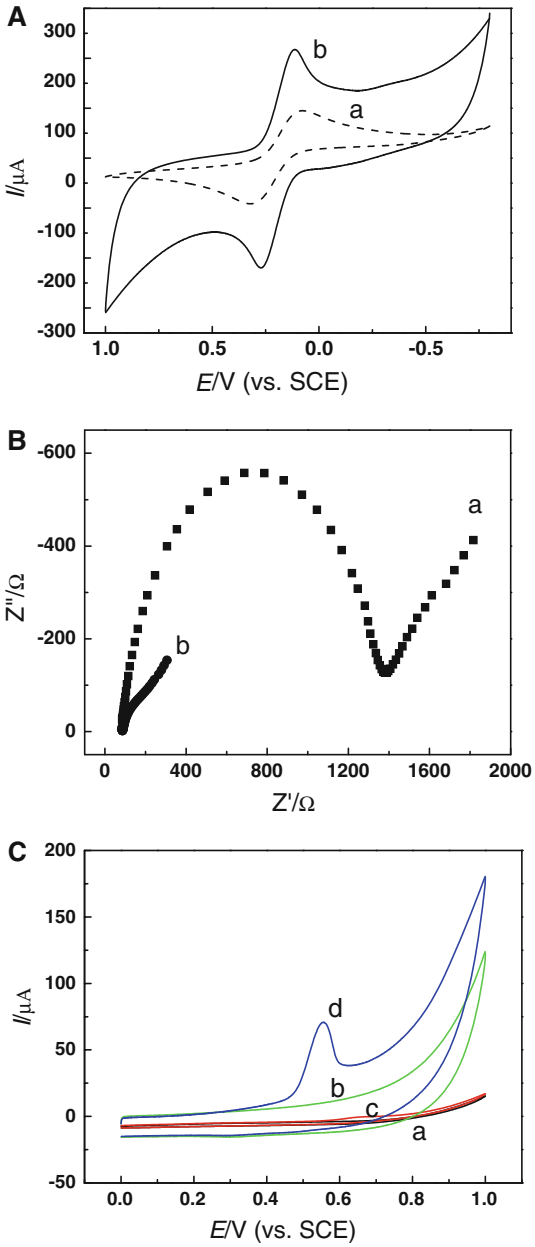
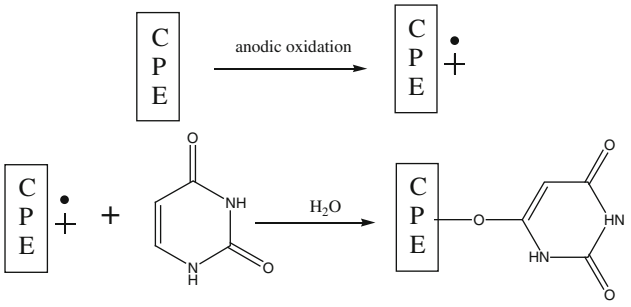


Fig. 1 CV (**A**) and EIS (**B**) of bare CPE (*a*), Ura/CPE (*b*) in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.1 M. CV (**C**) obtained at bare CPE (*a*, *c*) and Ura/CPE (*b*, *d*) in 0.1 M NaOH solution without (*a*, *b*) and with (*c*, *d*) 100 μM nevirapine



Scheme 2 The mechanism of uracil grafted on CPE

redox peaks were observed at bare CPE (a) and Ura/CPE (b) in the range of 0.0–1.0 V in NaOH solution without nevirapine. When 100 μM nevirapine was injected to NaOH, a broad oxidation peak was observed on bare electrode at 0.67 V (curve c). Compared with the bare CPE, the peak potential shifted negatively to 0.54 V and the oxidation peak was sharper on Ura/CPE, even more important the peak current enhanced almost 10-fold at Ura/CPE (curve d). The results indicated that uracil has

successfully grafted on electrode and greatly enhanced the sensitivity of electrode for nevirapine.

3.2 Optimization of experimental parameters

In order to further enhance the system performance, the experimental conditions including the concentration of uracil and accumulation time were investigated.

The uracil concentration employed in the electrodeposition process was found to have significant effects on the peak current of nevirapine. As shown in Fig. 2a, the oxidation peak currents increased significantly with the uracil concentration range of 0.1–1.0 mM. Nevertheless, the peak currents decreased when the concentration exceeded 1.0 mM, which may be ascribed to the thicker grafted film of uracil blocked the electrical conductivity. Consequently, 1.0 mM uracil solution was utilized to modify CPE.

The relationship between the oxidation peak currents and the accumulation time was also investigated. As shown in Fig. 2b, the currents increased rapidly in the range of 10–25 s and reached the maximum current response at 25 s, which indicated that nevirapine was adsorbed on the surface of Ura/CPE quickly. Then, the currents almost kept stable in spite of the increasing of time, demonstrating that the saturated adsorption of nevirapine at the surface of Ura/CPE was completed. In consequence, accumulation time of 25 s was selected for the determination of nevirapine.

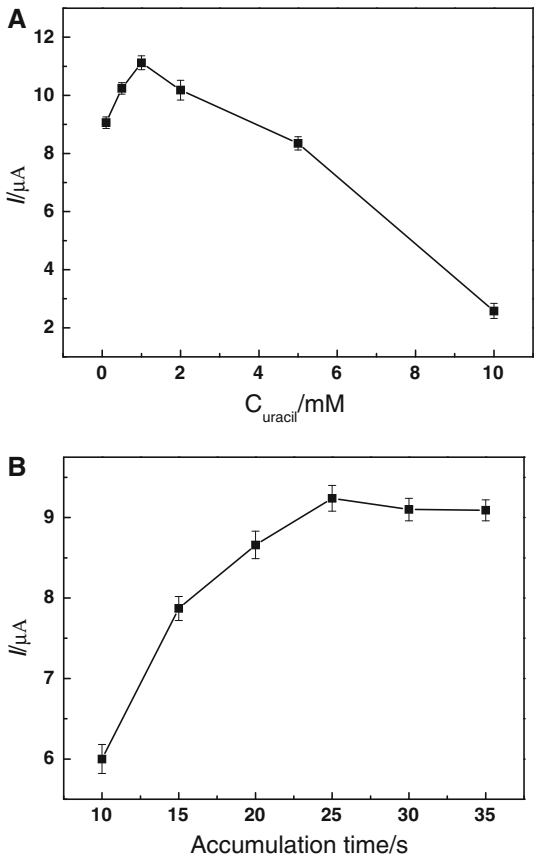


Fig. 2 **a** Effects of uracil concentration during the electro-deposition on the oxidation of 100 μM at the accumulation time of 25 s. **b** The influences of accumulation time on the oxidation current of 100 μM nevirapine at Ura/CPE prepared by electrodeposition of 1.0 mM uracil on electrode

3.3 Effect of pH

The effects of pH on electrochemical response of the Ura/CPE toward the detection of 100 μM nevirapine were investigated by DPV. Figure 3 displayed the effects of pH on the peak potentials and the peak currents of nevirapine in 0.1 M PBS with pH values ranging from 3–10 and in 0.1 M NaOH (pH 13) solution. It was found that the oxidation peak potentials shifted negatively and the peak currents enhanced with the increasing of pH, and reached most negative potential and highest current at pH 13. A linear relationship could be obtained between the peak potentials and solution pH with linear regression equation as follows: E_p (V) = 0.97 (V) − 0.038 pH. It indicated the participation of the same protons and electrons in the electrochemical process. The probable electrochemical reaction process for nevirapine oxidation at Ura/CPE could be summarized from references [31–33] and as shown in Scheme 3.

The hydroxide concentration for electrochemical response of Ura/CPE to nevirapine has been studied in series of NaOH concentrations ranging from 0.005 to 0.5 M, the maximum oxidation peak current of nevirapine was obtained at the concentration of 0.1 M. Therefore, 0.1 M NaOH was chosen as supporting electrolyte finally.

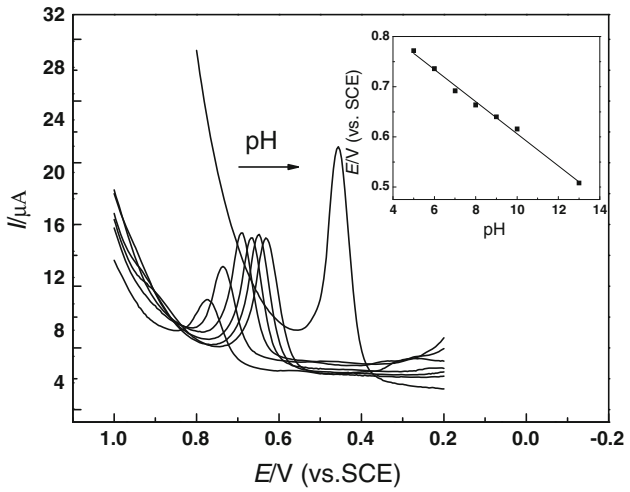
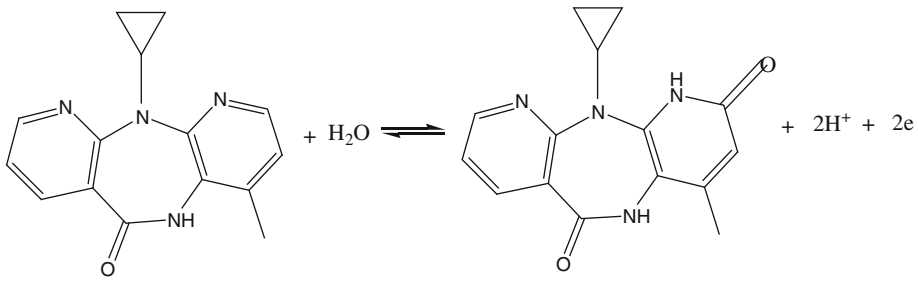


Fig. 3 Effects of pH on the oxidation peak current of 100.0 μM nevirapine at Ura/CPE performed by DPV in 0.1 M NaOH at the scan rate of 0.01 V s^{-1}

Scheme 3 The electrochemical oxidation mechanism of nevirapine on modified electrode



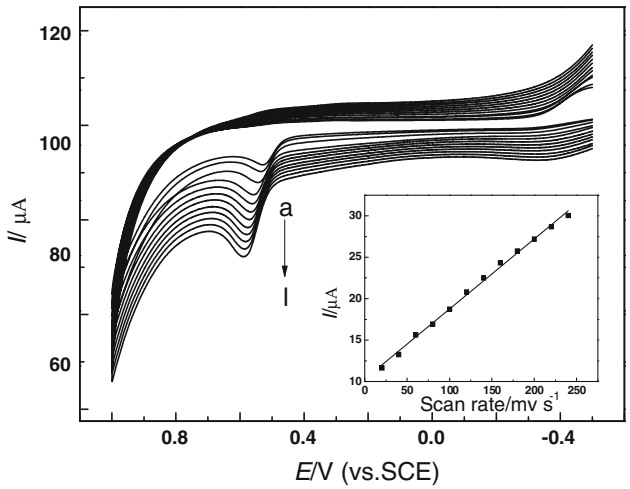


Fig. 4 The influences of scan rates (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 mV s^{-1}) on the oxidation current of 100.0 μM nevirapine in 0.1 M NaOH solution. *Inset graph* plots of peak currents versus scan rates

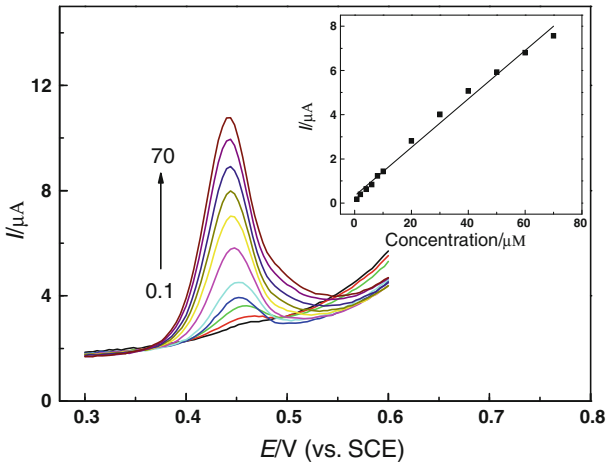


Fig. 5 Differential pulse voltammograms of nevirapine determination on Ura/CPE in 0.1 M NaOH solution. *Inset graph* The corresponding calibration curve of nevirapine with different concentrations

3.4 Mechanism of electrochemical determination

To investigate the determination mechanism of nevirapine on the Ura/CPE, the effect of scan rates on the oxidative reaction of 100.0 μM nevirapine was studied by CV. The cyclic voltammograms responses of Ura/CPE to nevirapine at various scan rates from 20 to 240 mV s^{-1} are exhibited in Fig. 4. The oxidation peak currents increased proportional to scan rates, suggesting that the electrochemical reaction of nevirapine on the modified electrode surface is an adsorption-controlled process, which is in consistent with the effect of accumulation time described in Sect. 3.2.

3.5 Calibration curve

Under optimized conditions, the electrochemical response of Ura/CPE for different concentrations of nevirapine was performed by differential pulse voltammetry. The results are shown in Fig. 5, the oxidation peak currents increased significantly with the rise of nevirapine concentration. The linearity between the oxidative peak current and nevirapine concentration was obtained in the range of 0.1–70.0 μM.

Equation can be described as follows: I_p (μA) = 0.33 + 0.11c (μM), with a correlation coefficient of 0.9951. The detection limit ($S/N = 3$) and sensitivity were calculated to be 0.05 μM and 2.073 μA mM^{-1} cm^{-2}, respectively.

Table 1 lists the proposed method compared with other methods reported for the determination of nevirapine [11–14, 16, 18]. As shown in Table 1, our method showed higher upper detection limit and wider concentration range than other reported papers. Therefore, owing to the incontestable advantage of electrochemical method, Ura/CPE will be a feasible electrochemical sensor for the testing of nevirapine.

3.6 Reproducibility, repeatability, stability, and interferences

The reproducibility of Ura/CPE was evaluated by DPV. The relative standard deviation (RSD) of the modified electrode response to 100.0 μM nevirapine for six successive measurements on the same electrode was 3.2 %. Furthermore, the current response of three Ura/CPE to 100.0 μM nevirapine were tested independently with a

Table 1 Comparison of major characteristics of various methods for the determination of nevirapine

Method	Dynamic ranges (μM)	Detection limits (μM)	References
High-performance liquid chromatography	0.2–39.0	0.2	[11]
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	0.04–3.8	0.04	[12]
Liquid chromatography–mass spectrometry	3.7–37.6	3.7	[13]
Micellar electrokinetic chromatographic	93.0–930.0	6.0	[14]
High-performance liquid chromatography–ultraviolet–visible spectroscopy	0.1–37.6	0.1	[16]
Adsorptive stripping voltammetry	0.04–0.5	0.003	[18]
Differential pulse voltammetry	0.1–70.0	0.05	Present work

RSD of 4.13 %, indicating a good repeatability. The stability of Ura/CPE was tested in the nevirapine solution intermittently. The catalytic current response to nevirapine maintained more than 90 % after the electrode stored dry at 4 °C in a refrigerator for 10 days.

Under optimized conditions, the influence of different interfering molecules for the determination of nevirapine was investigated on Ura/CPE. The results showed that the current response of nevirapine was unaffected even in the presence of 100-fold NaCl, KCl, $CaCl_2$, NaI, glucose, and sucrose, 10-fold concentration of thymine, uracil, tryptophan, tyrosine, ascorbic acid, paracetamol, and dopamine. But, the current response was affected in the presence of guanine and adenine, which may be owing to the direct reaction of uracil with guanine and adenine, as pyrimidine binds to purine via hydrogen bonds in RNA/DNA.

3.7 Analytical applications

In order to verify the applicability of the proposed sensor in clinical applications, Ura/CPE was utilized to detect nevirapine concentration in human serum samples. The results are shown in Table 2. It can be seen that the average recovery of nevirapine was between 99 % and 105 %. Moreover, the RSD was lower than 4 %, which showed a good precision of this method and met the requirement of microanalysis. Therefore, the developed method is applicable to the determination of nevirapine in clinical applications.

Table 2 Determination of nevirapine in synthetic samples (serum samples) ($n = 3$)

Number	Amount (μM)	Addition of nevirapine (μM)	Average recovery%	RSD%
1	15	5	98.5	1.5
2	20	5	102.1	3.1
3	25	10	97.7	2.2

4 Conclusions

In summary, a sensitive and selective sensor based on uracil-grafted CPE for the quantitative determination of nevirapine was developed. The modified electrode improved the electron transfer, enhanced the oxidation peak current of nevirapine, and decreased the oxidation overpotential obviously, indicating efficient catalytic activity in the detection of nevirapine. Under the optimum conditions (0.1 M NaOH, uracil concentration of 1 mM, accumulation time of 25 s), the modified electrode exhibited a variety of good electrochemical characteristics including low detection limit, high sensitivity, good selectivity, and favorable reproducibility. The present strategy provided a novel insight into the sensitive determination of nevirapine using only a modified electrode.

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